

Crowding induced entropy-enthalpy compensation in protein association equilibria

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A statistical mechanical theory is presented to predict the effects of macromolecular crowding on protein association equilibria, accounting for both excluded volume and attractive interactions between proteins and crowding molecules. Predicted binding free energies are in excellent agreement with simulation data over a wide range of crowder sizes and packing fraction. It is shown that attractive interactions between proteins and crowding agents counteract the stabilizing effects of excluded volume interactions. A critical attraction strength, for which there is no net effect of crowding, is almost independent of the crowder packing fraction.

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Protein-protein interactions are important in many essential biological functions, such as transcription, translation, and signal transduction [1]. A lot of progress has been made in understanding protein association in dilute solution via experiments and simulations [2–5]. Cells, on the other hand, contain various macromolecules, e.g., DNA, RNA, proteins, organelles, etc., which constitute up to 40% of the cell volume [6]. It is thus crucial to relate *in vitro* experimental or simulation results to those in a crowded cellular environment [7–13].

Several experimental studies have been performed to understand protein-protein interactions in a crowded environment [14–24]. Most attention has been paid to the steric excluded volume effects of inert crowding agents on the formation of protein complexes [25–27]. Very recent studies have also started to probe the effects of attractive interactions between proteins and crowders on protein association [28–31]. These studies have highlighted the importance of accounting for enthalpic effects arising from attractive interactions in addition to commonly invoked excluded volume effects. It was found that the enthalpic effects can actually increase the binding free energy (thereby destabilizing the bound complex) in contrast to predictions based on available theoretical models that can only capture entropic effects.

Most theoretical models of crowding are based on scaled particle theory (SPT) of hard-sphere fluids [32] or its modified versions and have been applied to interpret experimental and computational results with varying success. The failure of these models in several situations highlights an important role played by attractive crowder-protein interactions. Therefore, a need for comprehensive quantitative theory is warranted, that can describe the effects of repulsive as well as attractive crowder-protein interactions on the protein-association equilibria.

In this paper, we present a theory that can quantitatively predict the effects of macromolecular crowding on the protein association equilibria accounting for both repulsive and attractive crowder-protein interactions. The statistical mechanics and thermodynamics

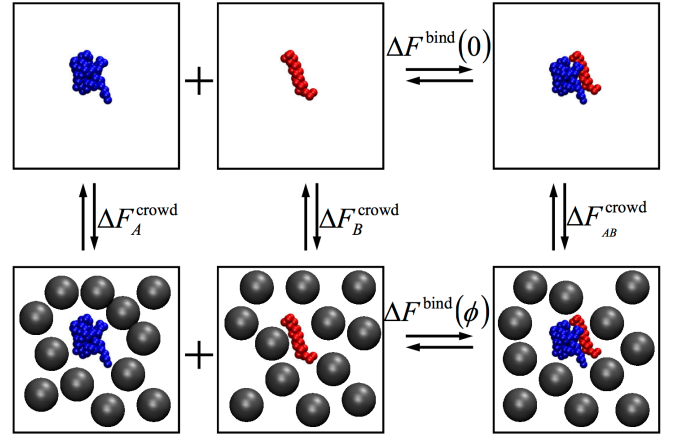


FIG. 1: Schematic diagram of the thermodynamic cycle for the formation of the Ubq/UIM1 complex. The ubiquitin is shown in blue while UIM1 is shown in red.

of a hard-sphere fluid are adapted to yield an approximate analytical expression for the protein-binding free energy in the presence of spherical crowders. Extensive replica exchange Monte Carlo (REMC) simulations have been performed on two distinct protein complexes to test this theory. We find that the theory is in excellent agreement with simulations over a wide range of crowder packing fractions and crowder-protein interactions. The theory identifies the region in parameter space (entropy-enthalpy compensation line in a two parameter plane) separating entropically stabilized area versus enthalpically destabilized one.

Theoretical development. Figure 1 illustrates a thermodynamic cycle that describes a change in the binding free energy ΔF^{bind} of two proteins due to the presence of crowding molecules. This change, $\Delta\Delta F^{\text{bind}}$, can be expressed as the difference in the binding free energy in the absence and presence of crowders and is given by

$$\begin{aligned}\Delta\Delta F^{\text{bind}}(\phi) &= \Delta F^{\text{bind}}(\phi) - \Delta F^{\text{bind}}(\phi=0) \\ &= \Delta F_{\text{AB}}^{\text{crowd}} - \Delta F_{\text{A}}^{\text{crowd}} - \Delta F_{\text{B}}^{\text{crowd}}, \quad (1)\end{aligned}$$

where $\Delta F_{\alpha}^{\text{crowd}}(\phi)$, ($\alpha \in [\text{A}, \text{B}, \text{AB}]$) is the solvation free

energy of a protein (or complex) α in a crowded solution with crowding packing fraction ϕ . [For brevity, we will omit the superscript “crowd” below.]

To obtain an expression for $\Delta F_\alpha(\phi)$ in Eq. (1) for a protein or complex α , let $U_\alpha(r, \Omega) = \sum_{i \in \alpha} u_i(r_i)$ be the overall interaction between a protein α and a crowder, where r is the distance between the center of mass of the protein and the crowder and Ω the orientational degree of freedom, while u_i is the interaction between an atom (or residue) i of the protein α and the crowder. For a general Lennard-Jones(LJ)-type potential for u_i , it is reasonable to assume that for given Ω , $U_\alpha(r, \Omega)$ exhibits a minimum, $-\epsilon_\alpha^m(\Omega)$, at $r = r_\alpha^m(\Omega)$. Following the Weeks-Chandler-Andersen (WCA) theory, we then decompose U_α into the repulsive and attractive parts as

$$\begin{aligned} U_{\alpha, \text{rep}}(r, \Omega) &= \begin{cases} U_\alpha(r, \Omega) + \epsilon_\alpha^m(\Omega) & r < r_\alpha^m(\Omega), \\ 0 & \text{otherwise,} \end{cases} \\ U_{\alpha, \text{att}}(r, \Omega) &= \begin{cases} -\epsilon_\alpha^m(\Omega) & r < r_\alpha^m(\Omega), \\ U_\alpha(r, \Omega) & \text{otherwise.} \end{cases} \end{aligned} \quad (2)$$

The solvation free energy, $\Delta F_\alpha(\phi)$, of the protein in a crowded solution can then be divided into two parts as,

$$\Delta F_\alpha(\phi) = \Delta F_{\alpha, \text{rep}}(\phi) + \Delta F_{\alpha, \text{att}}(\phi), \quad (3)$$

where $\Delta F_{\alpha, \text{rep}}(\text{att})$ is the contribution from the repulsive (attractive) interaction, respectively.

The repulsive contribution, $\Delta F_{\alpha, \text{rep}}$, is obtained by adopting the SPT. The SPT theory provides the free energy for solvating a hard-sphere of radius R_α in a bath of hard-sphere particles of radius R_c as,

$$\begin{aligned} \Delta F_{\alpha, \text{rep}} &= (3y + 3y^2 + y^3)\tilde{\phi} + (4.5y^2 + 3y^3)\tilde{\phi}^2 \\ &\quad + 3y^3\tilde{\phi}^3 - \ln(1 - \phi), \end{aligned} \quad (4)$$

where $\tilde{\phi} = \phi/(1 - \phi)$ and $y = R_\alpha/R_c$. But can we represent an anisometric protein with soft-core protein-crowder interactions as a hard sphere with an appropriate radius R_α to capture protein's solvation behavior accurately? Here we use the Boltzmann criteria to define R_α as,

$$\frac{4\pi}{3}(R_\alpha + R_c)^3 = \int_{U_{\alpha, \text{rep}} = f k_B T} r^2 dr d\Omega, \quad (5)$$

where the right-hand side represents the volume encompassed by the condition $U_{\alpha, \text{rep}}(r, \Omega) \geq f k_B T$. Here, we use $f = 2$ that has been used successfully in previous studies [33].

Using thermodynamic perturbation theory approach, the attractive contribution, $\Delta F_{\alpha, \text{att}}$, can be expressed as (up to the first order),

$$\Delta F_{\alpha, \text{att}} \approx \langle U_{\alpha, \text{att}} \rangle_{\text{rep}} = \int \rho U_{\alpha, \text{att}}(r, \Omega) g_0(r) r^2 dr d\Omega, \quad (6)$$

where ρ is the crowder number density related to ϕ via $\rho = \phi/(4\pi R_c^3/3)$, and $g_0(r)$ is the radial distribution function of the hard-sphere crowders between a protein and a crowder. Realizing that $g_0(r)$ has a maximum g_0^{max} at contact and then decays rapidly to unity, we assume $g_0(r) = g_0^{\text{max}}$ for $r \in [r_\alpha^m, r_\alpha^m + \lambda)$ and 1 for $r \in [r_\alpha^m + \lambda, \infty)$ with $\lambda = (2^{1/6} - 1)R_c \simeq 0.12R_c$ [34]. We then approximate Eq.(6) as,

$$\Delta F_{\alpha, \text{att}} \approx -\rho \bar{\epsilon}_\alpha S_\alpha \{\delta r + (g_0^{\text{max}} - 1)\lambda\}, \quad (7)$$

where $\bar{\epsilon}_\alpha = \langle \epsilon_\alpha^m \rangle_\Omega$ is the orientational average of ϵ_α^m , $S_\alpha = \int [r_\alpha^m(\Omega)]^2 d\Omega$ the surface area around the protein, and δr the attraction range. Note that here we assume $\delta r \geq \lambda$.

To enhance the simplicity and practical value of our theory, we use the Carnahan-Starling (CS) equation of state for a hard sphere fluid to calculate g_0^{max} . The CS equation of state is known to reproduce the thermodynamic behavior of hard-sphere fluids from dilute gas to near the freezing transition. The CS expression for g_0^{max} is given by

$$g_0^{\text{max}} = g_{\text{CS}}^{\text{max}}(\phi) = (1 - \phi/2)/(1 - \phi)^3, \quad (8)$$

and only depends on ϕ . Note that the first term in Eq. (7) gives a linear order in ϕ while the term containing g_0^{max} yields higher order terms. Combining together Eqs. (1), (3), (4), (7) and (8), one can easily obtain an estimate of crowding induced change in the binding free energy. Next, we test this theory against REMC simulations of two protein complexes in a wide range of crowder sizes, packing fractions and interaction strengths.

Model and simulation details. To proceed further, we first introduce our model and simulation procedure briefly. A residue-based coarse-grained model is used to simulate protein-protein interactions [35]. This transferable protein-protein interaction model was shown to yield binding affinities and structures for moderate-to-weakly interacting protein complexes in accord with experiments [35, 36]. Crowding agents are represented by spheres interacting via a repulsive potential, $u_{\text{rep}}(r) = \epsilon_r \left(\frac{\sigma_r}{r - 2r_c + \sigma_r} \right)^{12}$, where σ_r is the interaction range set equal to 6Å. The interaction between a residue i and a crowder is given by a modified LJ potential as,

$$u_i(r) = 4\epsilon_c \left[\left(\frac{\sigma_r}{r - \sigma_i + \sigma_r} \right)^{12} - \left(\frac{\sigma_r}{r - \sigma_i + \sigma_r} \right)^6 \right], \quad (9)$$

where $\sigma_i = \kappa_i + r_c$ with κ_i being the van der Waals radius of the residue i . This form of the potential, instead of the usual LJ form, ensures that the interaction range is independent of r_c . If the interaction between a residue i and a crowder is repulsive, we take $u_{\text{rep}}(r)$ with $2r_c$ replaced by σ_i .

Replica exchange Monte Carlo (REMC) simulations are performed on two distinct protein complexes, ubiquitin-UIM1 (Ubq/UIM1) and cytochrome *c*-cytochrome *c* peroxidase (Cc/CcP) following standard

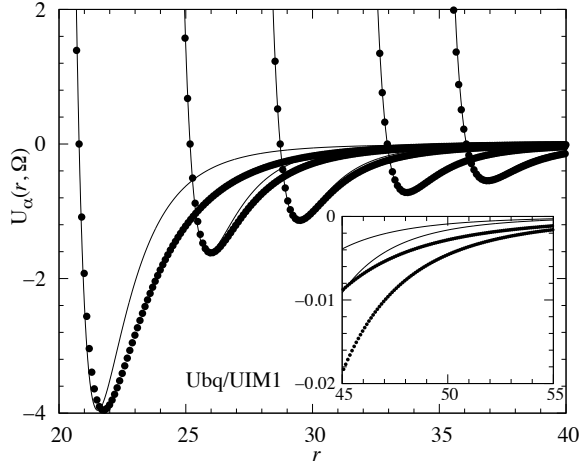


FIG. 2: Plot of the overall interaction between the Ubq/UIP1 complex and a crowder, $U_{\text{Ubq/UIP1}}$, as a function of r for different orientations Ω . The solid curves are obtained from Eq. (9) with $\epsilon_c = \epsilon_{\min}(\Omega)$ and $\sigma_i = r_0(\Omega)$.

protocols [35]. Repulsive as well as attractive interactions are considered for the residue-crowder interaction with $\epsilon_r = 1.69k_B T$ for the repulsive interaction, and $\epsilon_c = 0.15, 0.3, 0.45, 0.6k_B T$ for attractive interactions. A wide range of crowder size r_c and “bare” crowder volume fraction, $\phi_0 = 4\pi N_c r_c^3 / 3V$, are considered where N_c is the number of crowders and $V = L^3$ the volume of the simulation box of length L .

Results and Discussion. The spherical crowders interact with each other via the distance-dependent soft repulsive potential given by $u_{\text{rep}}(r)$ with a characteristic size r_c and $\epsilon_r = 1.69k_B T$. To apply the SPT theory (4) for calculating the repulsive contribution of the binding free energy, it is necessary to obtain an effective hard-sphere radius for such crowders. We define the effective hard-sphere radius, R_c , of crowders by the condition, $u_{\text{rep}}(2R_c) = f k_B T$ with the same f as in Eq. (5). This yields $R_c = r_c + \gamma \sigma_r$ where $\gamma = \frac{1}{2}[(\frac{1.69}{2.0})^{1/12} - 1]$. Note that although for $\epsilon_r = 1.69k_B T$ one has $R_c \simeq r_c$, in general, R_c can be different from r_c . The effective packing fraction ϕ is then given by $\phi = \phi_0 (R_c/r_c)^3$.

Figure 2 presents the overall interaction between the complex Ubq/UIP1 and a crowder at five different orientations, illustrating a highly anisotropic and asymmetric nature of the interaction. It shows that the overall protein-crowder interaction follows the LJ shape of the residue-crowder interaction, Eq. (9), (see the solid curves), with a minimum $-\epsilon_m(\Omega)$ at $r = r_m(\Omega)$ for a given Ω . However, the longer-distance tails are underestimated by the same formula as evident in the inset.

The effective radius, R_α , for a protein α , determined by Eq. (5) depends weakly on r_c and ϵ_c as shown in Table I. For the repulsive protein-crowder interactions, such effective radii for proteins and complexes are sufficient enough to calculate the change in the binding free en-

TABLE I: Effective radius, R_α , (in Å), for the ubiquitin (Ubq), UIP1 and the Ubq/UIP1 complex for $r_c = 12, 16, 20\text{\AA}$ for attractive ($\epsilon_c = 0.15, 0.3, 0.45, 0.6k_B T$) and repulsive (rep; $\epsilon_r = 1.69k_B T$) interactions

ϵ_c	Ubq			UIP1			Ubq/UIP1		
	12	16	20	12	16	20	12	16	20
0.15	14.13	14.39	14.57	9.82	10.14	10.37	15.88	16.17	16.38
0.30	14.29	14.54	14.72	9.99	10.31	10.54	16.03	16.32	16.53
0.45	14.36	14.61	14.79	10.07	10.38	10.62	16.11	16.39	16.60
0.60	14.41	14.65	14.83	10.12	10.43	10.66	16.15	16.44	16.64
rep	15.18	15.42	15.59	10.83	11.13	11.35	16.92	17.20	17.40

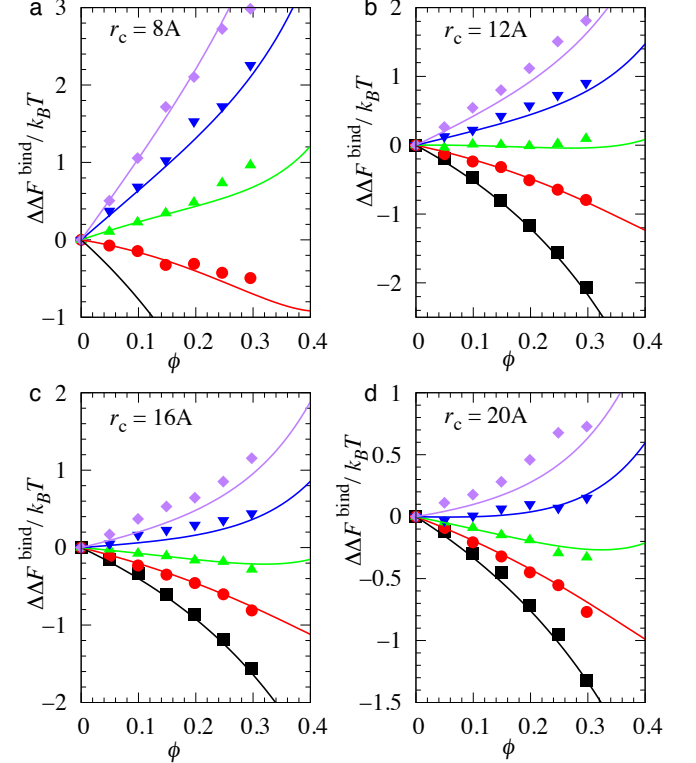


FIG. 3: Binding free energy, $\Delta\Delta F_b(\phi)$, for the Ubq/UIP1 complex as a function of the crowder packing fraction ϕ . The symbols are simulation data while solid curves are predictions from the theory (see the text).

ergy, $\Delta\Delta F^{\text{bind}}$, via Eq. (4). Figure 3 shows an excellent agreement between simulation results (black squares) and the theory (black solid curves) for the Ubq/UIP1 complex for different crowder sizes. As previously reported by us and others, the binding free energy decreases with increasing packing fraction ϕ and decreasing crowder size due to the excluded-volume effect.

Recent studies [28–30] have shown that attractive protein-crowder interactions can destabilize protein association. Figure 3 shows that *indeed* as the attraction strength, ϵ_c , between a residue and a crowder increases the binding free energy also increases with the packing fraction ϕ . For example, for a moderate strength

$\epsilon_c = 0.6k_B T$ the change in the binding free energy at $\phi = 0.3$ (close to the physiological condition) is up to about $4 k_B T$ when the protein-crowder interaction switches from repulsive (black) to attractive (purple). In order to apply our theory, Eqs. (1)-(8), to describe the simulation data for various ϵ_c and r_c , we calculate the average attraction strength, $\bar{\epsilon}_\alpha$, and the surface area, S_α , for the individual proteins and the complex. Note that $\bar{\epsilon}_\alpha$ is proportional to ϵ_c while S_α is independent of ϵ_c . Table II shows these values for different r_c . The theory predictions are in excellent agreement with the simulation data in which the attraction range $\delta r = 5 \text{ \AA}$ (close to σ_r) is used for all the crowder sizes and attraction strengths.

To check whether the theory can be transferable to other protein complexes, we calculate the binding free energies for the Cc/CcP complex (total 402 residues compared to 100 residues for the Ubq/UiM1 complex) as shown in Fig. 4. With the same δr , the theoretical predictions agree remarkably well with the simulation data.

The data in Figures 3 and 4 show the competition between entropic effects of the excluded volume and enthalpic effects by attractive crowder-protein interactions. As previously suggested [29, 30], the enthalpic effects can be approximated to be proportional to protein's surface areas and our theory here provides its concrete foundation from microscopic nature of the protein-crowder interactions. At high attraction strengths, the enthalpic penalty for breaking the crowder-protein interactions (at the expense of protein-protein interactions) dominates, thus increasing the binding free energy. At some critical attraction ϵ_c^{crit} , the two contributions are canceled out, and the binding energy in a crowded solution becomes equal to that in the absence of crowders (see green triangles and curve in Fig. 3b).

It was observed [30] that the critical attraction, ϵ_c^{crit} , for which the effect of the excluded volume is canceled out exactly by that of the attractive contribution, (i.e., $\Delta\Delta F^{\text{bind}} = 0$), is almost independent of the crowder packing fraction, ϕ . This is owing to the fact that $\Delta\Delta F^{\text{bind}}$ is almost linear in ϕ for ϵ_c considered. To obtain ϵ_c^{crit} estimate, we combine Eqs. (4) and (7) and solve for ϵ_c that satisfies $\Delta\Delta F^{\text{bind}} = 0$ up to the linear order

TABLE II: Normalized average attraction strength, $\bar{\epsilon}_\alpha/\epsilon_c$, and the surface area, S_α , (in \AA^3) for Ubq, UiM1 and the Ubq/UiM1 complex

r_c	Ubq		UiM1		Ubq/UiM1	
	$\bar{\epsilon}_\alpha/\epsilon_c$	S_α	$\bar{\epsilon}_\alpha/\epsilon_c$	S_α	$\bar{\epsilon}_\alpha/\epsilon_c$	S_α
8	4.56	6543	4.01	4100	4.61	7522
12	4.71	9278	4.07	6418	4.75	10487
16	4.79	12402	4.07	9143	4.85	13830
20	4.85	15921	4.04	12273	4.91	17562

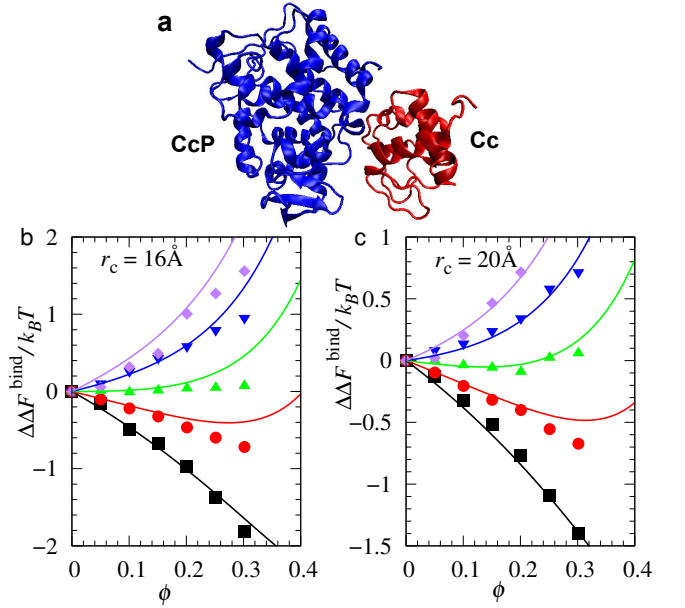


FIG. 4: Binding free energy, $\Delta\Delta F_b$, for the Cc/CcP complex as a function of ϕ . Symbols and curves are same as in Fig. 3.

in ϕ . One then obtains,

$$\epsilon_c^{\text{crit}} = \Delta Y / \Delta W + O(\phi^2), \quad (10)$$

where

$$\begin{aligned} \Delta Y &= 3(y_A + y_B - y_{AB}) + 3(y_A^2 + y_B^2 - y_{AB}^2) \\ &\quad + (y_A^3 + y_B^3 - y_{AB}^3), \\ \Delta W &= 3(\bar{\epsilon}_A S_A + \bar{\epsilon}_B S_B - \bar{\epsilon}_{AB} S_{AB}) \delta r / (4\pi R_c^3 \epsilon_c) \end{aligned} \quad (11)$$

This yields $\epsilon_c^{\text{crit}}/k_B T \simeq 0.19, 0.27, 0.36$ and 0.44 for $r_c = 8, 12, 16, 20 \text{ \AA}$, for the Ubq/UiM1, and 0.28 and 0.35 for $r_c = 16$ and 20 \AA for the Cc/CcP, respectively, consistent with simulation data in Figs. 3 and 4. We can also plot ϵ_c^{crit} as it changes with crowder size r_c as shown in Figure 5. For crowder-protein attraction values above this line, one will observe destabilization of protein association and stabilization below this line.

In summary, we have presented a quantitative theory for protein association equilibria in a crowded solution for both repulsive and attractive crowder-protein interactions. The theory is based on the statistical mechanics and thermodynamics of a hard-sphere fluid. Even though proteins are highly anisometric, the repulsive contribution to the binding free energy is described well by the scaled particle theory of hard spheres. The expression for the attractive contribution is obtained by using thermodynamic perturbation theory and the radial distribution function of hard-sphere fluids. The theory is in excellent agreement with simulation results for the Ubq/UiM1 and Cc/CcP complexes over a wide range of the crowder sizes, packing fractions and attraction strengths. Our work provides theoretical foundation of understanding

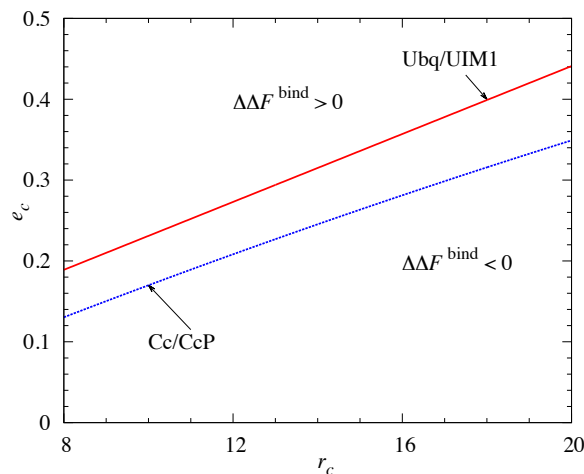


FIG. 5: Enthalpy-entropy compensation lines (i.e., $\Delta\Delta F^{\text{bind}} = 0$) in the parameter space (ϵ_c , r_c) for Ubq/UIM1 and Cc/CcP complexes.

the protein-protein interactions in a cellular environment in which proteins and crowding macromolecules exhibit non-specific interactions in addition to the excluded volume effects.

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